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Allosteric modulators for the A_1-adenosine receptor

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Allosteric modulators of endogenous adenosine represent an alternative to direct acting adenosine agonists and nucleoside uptake blockers. These compounds can selectively enhance the response to adenosine in only those organs or localised areas of a given organ in which production of adenosine is increased. The present article is an overview of the recent patent literature related to allosteric modulators of adenosine function on the A_1 receptor. In particular, the compounds with improved potency as enhancers and reduced antagonist properties are mentioned. Among the reported compounds, two molecules appear to be of potential therapeutic utility. A synergistic combination of PD-81723 and cyclopentyladenosine, an allosteric enhancer and agonist, respectively, of the adenosine A_1 receptor, appeared to be effective to induce angiogenesis. Moreover, (2-amino-4,5,6,7-tetrahydro-benzo[b]thiophen-3-yl)-(4-chloro-phenyl)-methanone appears to be active for the treatment of neuropathic pain without co-administration of adenosine.

Keywords: adenosine receptor, allosteric modulators, angiogenesis, cAMP content, neuropathic pain

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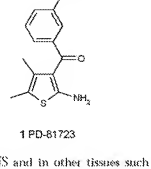
1. Introduction

Allosteric effects are observed when there are interactions between two binding processes that occur simultaneously or sequentially: the binding of one ligand affects the binding of another ligand. The flexible nature of the interaction between the receptors and various allosteric modulators, together with the potential for subtype selectivity, make allosteric sites attractive for therapeutic intervention [1-3]. In the case of the $GABA_A$ receptor, which is a transmitter-gated ion channel, the benzodiazepines acting on an allosteric site on the receptor showed substantial therapeutic effects and acceptable side effects [4].

Adenosine is a ubiquitous autocoid that modulates numerous functions in the cardiovascular and other organ systems. This local hormone exerts its actions on the human body by interacting with at least four different cell surface P_1-purinoreceptor subtypes classified as A_1, A_{2A}, A_{2B} and A_3 [5]. These receptor subtypes belong to the superfamily of G-protein-coupled receptors (GPCRs) and are widely distributed throughout the body [6,7]. Extracellular adenosine, as a breakdown product of ATP, protects tissues from ischaemic damage by lowering oxygen demand and increasing oxygen supply. Agents that increase the activation of the A_1-adenosine receptor in response to adenosine would be useful in conditions characterised by a localised oxygen deficit, such as angina, myocardial infarction and stroke [8].

The adenosine A_1 receptor is coupled to G_i/G_o protein signal transduction pathways and may mediate a number of biochemical processes, such as the activation of several types of K^+ channels, inactivation of N-, P- and Q-type Ca^{2+} channels, inhibition of adenylyl cyclase [9] and activation of phospholipase C. A variety of adenosine-mediated effects occur via the A_1-adenosine receptors, highly



1 PD-81723

expressed in the CNS and in other tissues such as kidney, lung, bladder and heart [6,7]. The widespread expression of adenosine A_1 receptors and the lack of sufficiently selective adenosine agonists have been major impediments to the successful development of direct-acting adenosine receptor agonists to exploit the cytoprotective properties of adenosine. Thus, it would be of great therapeutic importance to have compounds that are able to enhance the activation of A_1-adenosine receptors by the endogenous ligand, adenosine, within specific target tissues. Such an opportunity for intervention is provided by the concept of allosteric modulation of GPCRs [10].

Allosteric enhancers, upon binding, are believed to stabilise a conformation of the A_1-adenosine receptor that has a high affinity for agonists. This effect is manifested as a slowing of the rate of dissociation of agonist from the receptor [11]. In addition, an allosteric enhancer appears to stabilise an active conformation of the receptor even in the absence of an agonist. Thus, in cells with A_1-adenosine receptors that are active spontaneously in the absence of an agonist, such as the Chinese hamster ovary (CHO) cells used in numerous studies, an allosteric enhancer may increase the number of receptors that are active at any given time and thereby cause a change in cell function [12,13]. The currently available allosteric enhancers of agonist binding to the A_1-adenosine receptor have several nonspecific actions. These nonspecific actions include antagonism of the A_1-adenosine receptor [11,14] and inhibition of the activity of adenylyl cyclase [13].

Bruns and co-workers reported that 2-amino-3-benzoylthiophene derivatives are capable of enhancing both the binding and activity of reference A_1 receptor agonists, such as N^6-cyclopentyladenosine (CPA), to the A_1-adenosine receptor [14]. They also reported that these compounds were capable of acting as competitive antagonists at the same receptor, usually at higher concentrations [14]. Therefore, the concentration range where these compounds can enhance the effects of agonists is limited [11]. Among the compounds tested by Bruns, PD-81723 (2-amino-4,5-dimethylthien-3-yl)-[3-(trifluoromethyl)phenyl] methanone; compound **1**) represents a specific and selective allosteric enhancer of agonist binding to the A_1 receptor, with the best ratio of enhancement to antagonistic action at this receptor [12,15].

PD-81723 was shown to enhance agonist binding and the functional activation of the A_1 receptor in both brain [16] and cardiovascular tissues [17,18]. PD-81723 is selective for adenosine A_1 receptors, having no effects on other adenosine receptor subtypes or on other classes of receptors. While the exact molecular mechanism(s) through which PD-81723 exerts its allosteric actions remain unknown, the available data indicate that PD-81723 functions to stabilise a high affinity or agonist-preferring state of the A_1 receptor [19-21].

Compound PD-81723 was claimed to be useful for treating conditions in which angiogenesis is involved [101] and this effect was due to a synergism between an adenosine A_1 receptor agonist and PD-81723. The combination of low dose CPA with PD-81723 resulted in stimulation of angiogenesis, that was threefold greater than that seen with PD-81723 alone. Promoting angiogenesis is beneficial for revascularisation of ischaemic tissues in conditions such as stroke, heart disease and peripheral vascular disease [22]. In the Univ. of Virginia patent [101] it was demonstrated that PD-81723 stimulated angiogenesis in two animal model systems, the chicken chorioallantoic membrane (CAM) model [23] and the rat mesenteric model [24,101]. This data indicates that the A_1 receptor plays an important role in angiogenesis that was previously unknown. The reason that this effect is clinically important is because allosteric enhancers would theoretically be most effective in hypoxic tissues with high endogenous levels of adenosine, sparing other tissues from angiogenic effects where they are not needed.

2. Molecules that allosterically interact with the adenosine A_1 receptor

To study the role of various substitutions on the phenyl ring and the importance of the 4,5-dimethyl group on the thienyl ring, Bruns [14], Baraldi [25,102], Ijzerman [26,27], Tranberg [28] and Lütjens [29] have described the synthesis and biological evaluation of compounds useful as potent, yet selective, allosteric enhancers of the A_1-adenosine receptor. It was evident from previous structure–activity relationship (SAR) studies [14,25-29] that substitution with electron-withdrawing substituents, such as chlorine and trifluoromethyl, on the benzoyl moiety at the 3-position of the thiophene ring resulted in higher enhancement activity.

Many of the tested compounds reported in the patent filed by Medco Res. (now King Pharm. Res. & Dev., Inc.) in 1997 [102] and represented in Figure 1, appeared to be enhancers (e.g., most compounds with general formula **2** and **3**), whereas others appeared to be antagonists of the A_1-adenosine receptor (e.g., most compounds with general formula **4**). Although none of the tested compounds had greater efficacy than PD-81723, compounds **2d**, **2g**, **2j** and **3a** appeared to be comparable to PD-81723 as enhancers at all concentrations tested. Since the known allosteric enhancers are also A_1-adenosine receptor antagonists at some (usually high) concentrations, the fact that none of

the tested compounds had a greater efficacy than 10 μM PD 81723 could be explained by a potential antagonistic effect of the compounds at high concentrations.

In the series of compounds with general formula **2**, the structure of PD 81723 has been systematically modified. For the 4,5-dimethyl thiophene derivatives (R_1 = R_2 = CH_3), the absence of the trifluoromethyl moiety on the phenyl ring (compound **2a**, R_3 = R_4 = R_5 = H), led to a loss of activity at any concentration, while removing both methyl substituents in positions 4 and 5 on the thiophene ring (compound **2b**, R_{1-5} = H), led to an increase of activity at a lower concentration (0.1 μM). However, this latter derivative was less active than PD 81723 at 1 and 10 μM. No increase of activity was obtained with the presence of a non-polar substituent (such as chloro) in the *para*-position on the benzoyl ring (compound **2c**). In the series of 4,5-dimethyl thiophene derivatives reported in the patent filed by Medco Res. [102], only compound **2d** possessed an activity comparable with that of PD 81723 at each concentration tested.

The effect of substitutions at the 4- and 5-positions of the thiophene ring in the PD 81723 and particularly on its 'cyclised' analogues, has been carefully examined [25,29]. The bridging of the 4- and 5-positions with a methylene chain, [-$(CH_2)_n$-], with n = 3, to give compound **2e**, caused an increase of activity compared to that reported for the corresponding 4,5-dimethyl derivative **2a** at 10 μM, but the potency was lower with respect to that described for PD 81723. When the methylene in position 6 of the dihydrocyclopentadien[b]thiophene ring of compound **2e** was replaced with a sulfur atom (compound **2f**), a loss of activity at 0.1 and 1 μM was observed. However, at 10 μM this latter compound was only slightly less effective than PD 81723.

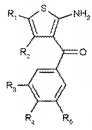
A large number of compounds have been reported in the series of 'cyclised' PD 81723 analogues. These are represented by the dihydrocyclopentadien[b]thiophene derivatives structurally related to **2e** and modified on the 3-benzoyl moiety. As was just noted, compound **2e** is less active as an enhancer than PD 81723. Application of the 'Topliss' approach [30] to this series, by varying substituents on the benzoyl moiety, showed that the addition of a halogen, especially a bromine (compound **2g**), was preferred over a methoxy (compound **2h**) or nitro (compound **2i**) substituent at position 4 of the benzoyl moiety. The 4,5,6,7-tetrahydrobenzo[b]thiophene derivatives **2j** – **2l** also showed good allosteric enhancer activity and appeared to be more potent than PD 81723 at lower concentrations (0.1 and 1 μM).

Comparing the activity of the homologous series **2e**, **2j**, **2m** and **2n**, where the benzoyl moiety is maintained constant, it appears that activity is associated with the size of the ring fused to the thiophene. By increasing the size of this ring from five carbons (compound **2e**) to six carbons (compound **2j**), a significant increase in activity is observed. A further increase to seven carbons (compound **2m**) or eight carbons (compound **2n**) leads to a modest decrease in activity, with no significant difference between the two.

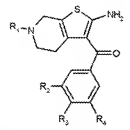
Compound **2j** was specifically claimed by King [103] to be useful for the treatment of a pathologically hyper excited sensory nerve function in a conscious human subject and disorder treatment includes hyperesthesia, dysesthesia, allodynia, hyperalgesia, tinnitus, ganglionic dysfunction and combinations thereof. These symptoms are often manifested as neuropathic pain [104], which is a persistent, chronic pain usually described as a burning, shooting or lancinating sensation without an obvious cause.

King Pharm. has also claimed a series of compounds, in which the C-6 methylene group was replaced with a nitrogen atom to give the 4,5,6,7-tetrahydrothieno[2,3-c]pyridine derivatives with general structure **3** [105,106]. In the series of compounds that bear the 3-trifluoromethyl substituent of PD 81723 and different aralkyl moieties (R_1 substituent) on position six, the benzyl moiety was the best substituent. Indeed, only the N-benzyl derivative **3a** possessed an activity comparable to PD 81723 at a concentration of 10 μM, while compounds characterised by the presence of an N-[2-(phenyl)ethyl] (compound **3b**) or N-[3-(phenyl)propyl] (compound **3c**) moiety were inactive. The replacement of the 3-trifluoromethyl with a 4-chloro substituent on the benzoyl group of compound **3a** (compound **3d**) led to a complete loss of activity. The removal of the N-benzyl group (compound **3e**) or both the N-benzyl and 4-chloro substituents (compound **3f**) did not restore activity. It has been reported by Bruns [14] that a positive charge on the nitrogen reduces enhancing activity. This is substantiated by the N-ethoxycarbonyl derivatives **3g** and **3h** that show a very potent enhancing activity. Further confirmation of Bruns' observation was obtained by the synthesis of the N-ethoxycarbonyl methyl homologues (R_1 = $CH_2CO_2C_2H_5$) of compounds **3g** and **3h** (compounds **3i** and **3j**, respectively), wherein a methylene spacer has been inserted between the nitrogen and the ethoxycarbonyl moiety. These compounds contain a positively charged nitrogen at physiological pH and had no enhancing activity. The substitution of the ethoxycarbouyl moiety of compounds **3g** and **3h** with the N-benzyloxycarbonyl protective group (Z) (compounds **3k** and **3l**) reduced enhancing activity. Those compounds of general formula **4** that have been exemplified [106] were devoid of allosteric enhancer activity at all concentrations tested.

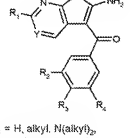
The results reported in **Table 1** [25] are generally consistent with the results reported by Bruns [14] and Hjerrman [26,27], although different assays and different sources of adenosine receptors were used in these studies. However, it should be noted that the assay of effects of putative enhancers on cAMP content of CHO cells expressing human A_1-adenosine receptors is not a specific assay of allosteric enhancement of agonist binding. This assay does not directly measure the interaction between receptor activation and G protein activation and these observations may be complicated by drug actions not related to enhancement, such as cell toxicity. However, the effect of a tested compound in the intact cell cAMP assay may



2a $R_1 = R_2 = CH_3$, $R_3 = R_4 = R_5 = H$
2b $R_1 = R_2 = R_3 = R_4 = R_5 = H$
2c $R_1 = R_2 = H$, $R_3 = R_5 = H$, $R_4 = Cl$
2d $R_1 = R_2 = CH_3$, $R_3 = R_5 = H$, $R_4 = Cl$
2e R_1, $R_2 = -(CH_2)_3-$, $R_3 = R_4 = R_5 = H$
2f R_1, $R_2 = -SCH_2CH_2-$, $R_3 = R_4 = R_5 = H$
2g R_1, $R_2 = -(CH_2)_3-$, $R_3 = R_5 = H$, $R_4 = Br$
2h R_1, $R_2 = -(CH_2)_3-$, $R_3 = R_5 = H$, $R_4 = OCH_3$
2i R_1, $R_2 = -(CH_2)_3-$, $R_3 = R_5 = H$, $R_4 = NO_2$
2j R_1, $R_2 = -(CH_2)_4-$, $R_3 = R_5 = H$, $R_4 = Cl$
2k R_1, $R_2 = -(CH_2)_4-$, $R_3 = R_5 = H$, $R_4 = Br$
2l R_1, $R_2 = -(CH_2)_4-$, $R_3 = R_5 = H$, $R_4 = p\text{-}C_6H_5$
2m R_1, $R_2 = -(CH_2)_5-$, $R_3 = R_5 = H$, $R_4 = Cl$
2n R_1, $R_2 = -(CH_2)_6-$, $R_3 = R_5 = H$, $R_4 = Cl$



3a $R_1 = Bn$, $R_2 = CF_3$, $R_3 = R_4 = H$
3b $R_1 = PhCH_2CH_2$, $R_2 = CF_3$, $R_3 = R_4 = H$
3c $R_1 = PhCH_2CH_2CH_2$, $R_2 = CF_3$, $R_3 = R_4 = H$
3d $R_1 = Bn$, $R_2 = R_4 = H$, $R_3 = Cl$
3e $R_1 = H$, $R_2 = R_4 = H$, $R_3 = Cl$
3f $R_1 = H$, $R_2 = R_3 = R_4 = H$
3g $R_1 = CO_2C_2H_5$, $R_2 = R_3 = R_4 = H$
3h $R_1 = CO_2C_2H_5$, $R_2 = R_4 = H$, $R_3 = Cl$
3i $R_1 = CH_2CO_2C_2H_5$, $R_2 = R_3 = R_4 = H$
3j $R_1 = CH_2CO_2C_2H_5$, $R_2 = R_4 = H$, $R_3 = Cl$
3k $R_1 = C_6H_5CH_2OCO$, $R_2 = R_3 = R_4 = H$
3l $R_1 = C_6H_5CH_2OCO$, $R_2 = R_4 = H$, $R_3 = Cl$



4 R_1 = H, alkyl, N(alkyl)$_2$, substituted alkyl, OH or NH_2
Y = CH, N, C-CN or C-C(O)OX, wherein X = H, alkyl or substituted alkyl
R_2, R_3 and R_4 are independently H, halogen, alkyl, substituted alkyl, aryl, amino, trifluoromethyl, nitro or cyano

Figure 1. General structures of 2-amino-3-benzoyl thiophenes.

Table 1. Percentage change in CHO cell cAMP content in the presence of tested compounds 2a – n and 3a – f.

Compound	Change in cAMP content from control at different concentrations of tested compound.		
	0.1 µM	*1 µM*	*10 µM*
1 (PD-81723)	-18 ± 4	-30 ± 6	-57 ± 11
2a	-2 ± 0	+3 ± 1	-11 ± 0
2b	-29 ± 1	-15 ± 3	-16 ± 3
2c	-4 ± 6	-5 ± 1	-14 ± 2
2d	-17 ± 6	-30 ± 8	-52 ± 13
2e	-13 ± 4	-10 ± 3	-30 ± 4
2f	+2 ± 0	-3 ± 1	-24 ± 5
2g	-18 ± 5	-30 ± 3	-41 ± 9
2h	+7 ± 2	-15 ± 2	-15 ± 3
2i	+3 ± 1	-8 ± 1	-5 ± 1
2j	-9 ± 2	-27 ± 6	-44 ± 9
2k	-18 ± 5	-24 ± 6	-24 ± 5
2l	-5 ± 1	-28 ± 8	-24 ± 6
2m	-9 ± 2	-15 ± 3	-26 ± 7
2n	-9 ± 3	-14 ± 4	-24 ± 5
3a	-10 ± 2	-23 ± 5	-48 ± 9
3b	-2 ± 1	-4 ± 1	+10 ± 3
3c	-3 ± 1	-4 ± 1	-12 ± 4
3d	-3 ± 1	+8 ± 3	+ 12 ± 3
3e	-9 ± 3	-7 ± 2	-5 ± 2
3f	-2 ± 0	-5 ± 2	-7 ± 2

CHO: Chinese hamster ovary.

be a more useful predictor of the effect of the compound *in vivo* than is a binding assay that more specifically assesses allosteric enhancement.

It was evident from the SAR studies that substitution with electron-withdrawing substituents, such as chloro and trifluoromethyl, on the benzoyl moiety at the 3-position of the thiophene ring resulted in higher enhancement activity. Compounds **2d**, **2g**, **2j** and **3a** appeared to be nearly as efficacious as PD 81723 and derivatives **2b**, **2d**, **2g** and **2k** caused significant reductions of cAMP content of CHO:hA_1 cells at a concentration of 0.1 µM. The dual actions of benzoylthiophenes to both antagonise and enhance the actions of A_1-adenosine receptor agonists at different concentrations has been noted previously [11].

In another patent application filed by King [137] the synthesis and biological evaluation of the allosteric enhancer activity of a new series of derivatives of PD 81723 with general formulae **5** – **8** (**Figure 2**) was reported. In these compounds, various modifications, both on a naphthalene ring and on the

4- and 5-position of the thiophene system, were studied to establish the structural requirements and the SARs for enhancement of the action of an agonist at the human A_3 adenosine receptor [28,31]. Previous studies by Brans [34] suggested that the addition of a fused ring on the thiophene provided improved allosteric enhancer activity. King sought to examine if this trend would also be observed with the more complex naphthoyl derivatives. Moreover, in the same series of 2-amino-3-benzoylthiophenes, the receptor environment adjoining the benzoyl binding site was known to be lipophilic. For this reason, the substituents at the 4-position on the naphthalene were varied from H, to groups with a different degree of lipophilicity (CH_3, CH_3O, Cl, Br and I) and at the 6-position from H to Cl. For substituents at the 4- and 5-position of the thiophene, King examined the 4,5-dimethyl and various alkylene linkages between the 4- and 5-positions. These linkages varied from three to four methylenes to yield the 5,6-dihydro-4H-cyclopentadien[b]thiophene and 4,5,6,7-tetrahydrobenzo[b]thiophene derivatives, respectively (compounds 5e – 5i and 6). Finally, by introducing a nitrogen atom into the 6-position of the tetrahydrobenzo[b]thiophene ring, the 4,5,6,7-tetrahydrothieno [2,3-c]pyridine derivatives 7 and 8 were prepared.

The reference compound for comparison was PD 81,723 (compound 1) and assays of allosteric enhancement were performed using CHO cells stably transfected to express the recombinant human A_1-adenosine receptors. Allosteric enhancement was measured as the ability of the compounds with general formulae 5 – 8 to reduce the cAMP content of CHO:hA_1 cells at four different concentrations (0.01, 0.1, 1 and 10 µM; Table 2).

Several molecules appeared to be more potent than PD 81,723 as allosteric enhancers in the CHO:hA_1 assay. These included compounds 5a – i, 6a and 8a. These latter compounds meet the following criteria: i) they cause a decrease of cAMP content of CHO:hA_1 cells by > 40% when present at a concentration of 10 µM; ii) they cause a decrease of cAMP content of ≥ 22% at a concentration of 1 µM; iii) they do not increase the content of cAMP of CHO:hA_1 cells at any concentration and i.v.) the response to the compound is concentration-dependent.

In the series of derivatives characterised by the presence of the unsubstituted 1-naphthoyl moiety at the 3-position of the thiophene ring and which differ in the R_1- and R_2-substituents, it appeared that the most potent compounds had a 3- or 4 carbon methylene linkage between the 4- and 5-position of the thiophene (compounds 5e and 5j, respectively).

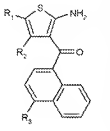
Several chemically different substituents at the 4-position of the 1-naphthoyl moiety were investigated. These modifications will alter the electronic, steric and lipophilic features of this residue. Generally, by the introduction of a lipophilic and electron-releasing methyl group at the 4-position of the naphthoyl ring, the allosteric enhancing activity was increased for compound 5a and 5e with respect to the reference compound PD 81,723 at any concentration. The replacement of the methyl with a less lipophilic and more electron releasing moiety (a methoxy group), reduced allosteric enhancer activity. For this latter compound, the reduction in activity may be attributed both to steric and electronic factors, as has been observed previously for the methoxy-benzoyl counterpart 2h [14,25]. In fact, the methoxy group has an angularity component, part of the methoxy group can extend significantly above or below the plane of the naphthalene ring, thus introducing a unique steric parameter.

In the same series of compounds represented by 5 and 7, replacement of the methyl group with substituents showing similar electronic effects but different lipophilic character (e.g., Cl, Br and I) resulted in improved activity (5b – d, 5g – i and 5k – l). It is noteworthy that although the introduction of a chlorine at the 4-position of the 1-naphthoyl moiety (compounds 5b and 5g) did not have a profound effect on activity relative to the 4-unsubstituted derivatives, increasing the size of the halogen atom from chlorine to bromine (derivatives 5c, 5h and 5k) and ultimately iodine (compounds 5d, 5i and 5l) caused a large increase of the allosteric enhancer activity, especially when a lipophilic substituent (methyl or cycloalkyl moiety) was present in the 4- and 5-position of the thiophene ring.

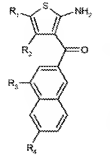
These findings suggest that the introduction of a lipophilic halogen or methyl group at the 4-position of the naphthalene ring was preferred for enhancing activity, whereas a more hydrophilic group, such as the methoxy, was not favourable. The results indicate a significant qualitative direct correlation between the allosteric enhancer activity and the lipophilicity of the substituent at the 4-position of the 1-naphthoyl moiety, with the compounds characterised by the presence of substituents with higher lipophilic character (Cl, Br, I) among the most potent as A_1-adenosine allosteric enhancers.

By examining regioisomers of various compounds with the same substituents, it has been possible to further elucidate the role of lipophilicity in the SARs associated with these molecules. This is exemplified by two groups of regioisomers, composed of thiophene derivatives substituted in the 3-position with the 1-naphthoyl or 2-naphthoyl moieties (compounds with general formulae 5 and 6, respectively). By comparing the activities of derivatives bearing the same substituents on the 4- and 5-position of the thiophene ring, it may be concluded that the 1-naphthoyl derivatives (general formula 5) are generally more potent than the corresponding analogues with the 2-naphthoyl substituent (general formula 6). We can further conclude that appropriate lipophilicity is a necessary parameter for the allosteric enhancer activity but not the only determining parameter because molecules with equivalent lipophilicity may have very different levels of activity.

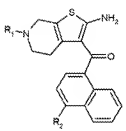
In the series of 2-naphthoyl derivatives with general formulae 6 and 8, only the compounds 6a and 8a are more active than PD 81,723. For derivative 8a, the introduction of



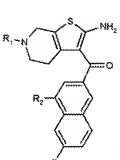
5a $R_1 = R_2 = CH_3$, $R_3 = CH_3$
5b $R_1 = R_2 = CH_3$, $R_3 = Cl$
5c $R_1 = R_2 = CH_3$, $R_3 = Br$
5d $R_1 = R_2 = CH_3$, $R_3 = I$
5e $R_1, R_2 = -(CH_2)_3-$, $R_3 = H$
5f $R_1, R_2 = -(CH_2)_3-$, $R_3 = CH_3$
5g $R_1, R_2 = -(CH_2)_3-$, $R_3 = Cl$
5h $R_1, R_2 = -(CH_2)_3-$, $R_3 = Br$
5i $R_1, R_2 = -(CH_2)_3-$, $R_3 = I$
5j $R_1, R_2 = -(CH_2)_4-$, $R_3 = H$
5k $R_1, R_2 = -(CH_2)_4-$, $R_3 = Br$
5l $R_1, R_2 = -(CH_2)_4-$, $R_3 = I$



6 $R_1 = R_2$ where R_1, R_2 = H, CH_3
$R_1, R_2 = -(CH_2)_n$
with n = 3, 4, 5 or 6
R_3 independently = H, halogen, alkyl, substituted alkyl, aryl, amino, trifluoromethyl, nitro or cyano
R_4 = H or halogen
6a $R_1, R_2 = -(CH_2)_3-$, $R_3 = R_4 = H$



7 R_1 = H, alkyl, (CH_2)n-aryl with n = 1, 2 or 3, CO-NH-alkyl, CO_2CH_2Ph, $S(O)_2$-alkyl, amino acid residue, $(CH_2)_nCO_2X$ with X = alkyl or aryl
R_2 = H, halogen, alkyl, substituted alkyl, aryl, amino, trifluoromethyl, nitro or cyano



8 R_1 = H, alkyl, $(CH_2)_n$-aryl with n = 1, 2 or 3, CO-NH-alkyl, CO_2CH_2Ph, $S(O)_2$-alkyl, amino acid residue, $(CH_2)nCO_2X$ with n = 0 – 20, X = alkyl or aryl
R_2 = H, halogen, alkyl, substituted alkyl, aryl, amino, trifluoromethyl, nitro or cyano
R_3 = H or halogen
8a R_1 = Bn, R_2 = H
8b R_1 = Bn, $R_2 = CH_3$, R_3 = Cl

Figure 2. General structures of 2-amino-3-naphthoyl thiophenes.

different electron-withdrawing, electron-donating and hydrophobic substituents on the phenyl ring of the N-benzyl moiety led to a marked decrease in their activity as A_1 allosteric-enhancers.

In the series of derivatives with general formulae 6 and 8 that possess the 4-methyl-2-naphthoyl moiety, the introduction of a lipophilic chlorine atom at the 6-position of the naphthalene moiety (compound 8b) did not improve the allosteric enhancer potency, which indicates an unfavorable steric interaction originating from the presence of 6-chloro substitution.

In the same patent application filed by King [107], the synthesis of compounds bearing a heteroaroyl instead of the benzoyl moiety at the 3-position of the thiophene were described (Figure 3, formulae 9 – 12). The heteroaroyl groups were selected to possess heteroatoms capable of forming hydrogen bonds within the binding domain (2-furanyl, 2-benzofuranyl and 2-pyridyl moieties in compounds with general formulae 9 and 10, respectively). In addition, especially with pyridine (compound 10), the hydrophilic properties of the molecules were increased, since low water-solubility is one of the major limitations of the 2-amino-3-benzoylthiophene derivatives 9. Unfortunately, the derivatives of general formulae 9 and 10, which potentially could form a hydrogen bond with the allosteric site of the A_1-adenosine receptor, were significantly less active.

Replacement of the benzoyl group with an isosteric/isoelectronic thiophene-carbonyl was generally well-tolerated. Compounds of general formulae 11 and 12, characterised

Table 2. Percentage change in CHO cell cAMP content in the presence of compounds 5a – 5l, 6a and 8a.

Compound	Change in cAMP content from control (mean ± SEM) at different concentrations of tested compound.			
	0.01 µM	*0.1 µM*	*1 µM*	*10 µM*
1 (PD-81723)	-1 ± 2	-7 ± 2	-13 ± 1	-50 ± 1
5a	-5 ± 1	-8 ± 3	-32 ± 2	-51 ± 4
5b	12 ± 4	-18 ± 5	-47 ± 6	-56 ± 2
5c	-8 ± 4	-1 ± 4	-2 ± 3	-60 ± 3
5d	-5 ± 6	-3 ± 10	-19 ± 10	-63 ± 5
5e	-0.6 ± 3	-6 ± 1	-29 ± 4	-60 ± 1
5f	9 ± 4	4 ± 3	-17 ± 4	-48 ± 2
5g	-10 ± 2	-15 ± 4	-27 ± 2	-55 ± 2
5h	3 ± 3	19 ± 5	-14 ± 5	-67 ± 3
5i	-6 ± 3	-8 ± 3	-22 ± 4	-75 ± 1
5j	-11 ± 4	-15 ± 4	-22 ± 3	-52 ± 3
5k	-6 ± 3	-10 ± 2	-29 ± 2	-72 ± 1
5l	-3 ± 4	-4 ± 4	-24 ± 5	-67 ± 2
6a	-19 ± 3	-14 ± 4	-15 ± 3	-51 ± 3
8a	-11 ± 4	-12 ± 3	-18 ± 3	-60 ± 4
8b	-1 ± 3	1 ± 4	-5 ± 3	-9 ± 3

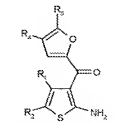
CHO: Chinese hamster ovary; SEM: Standard error of the mean.

by the presence of a 3- or 2-thiophene-carbonyl moiety, respectively, led to compounds with activity comparable to that of PD-81723 at a concentration of 10 µM and better than PD-81723 at lower concentrations. The position at which thiophene is connected to the carbonyl does not seem important, since 3-thienyl derivatives with general formula 11 possessed the same activity as the 2-thienyl regioisomers 12. To further increase the lipophilicity of these moieties and further probe the size of this hydrophobic domain, the introduction of an halogen (chlorine or bromine) at the 5-position of the thiophene-2-carbonyl moiety was also evaluated (compound 12). These compounds all proved to be inactive, with no significant difference between chloro- and bromo-derivatives, suggesting that a halogen is not tolerated in that position.

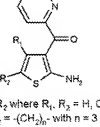
3. Expert opinion and conclusions

Most of the allosteric enhancers based on the structure of PD-81723 (compound 1) have been patented by King Pharm. Res. & Dev., Inc. or its predecessor, Medco Res.. The research focused on the preparation of compounds characterised by a reduced antagonist activity, a possible cause of side effects. The compounds are claimed for uses including protection against hypoxia, ischaemia-induced injury and treatment of adenosine-sensitive cardiac arrhythmias. The derivative (2-amino-4,5,6,7-tetrahydro-benzo[b]thiophen-3-yl)-(4-chloro-phenyl)-methanone (compound 2j) normalised hyperexcited sensory nerve functions in a model of neuropathic pain. The combined action of a selective adenosine A_1 allosteric enhancers (PD-81723) and a selective A_1 agonist (CPA) was claimed to induce angiogenesis at a desired location for treating conditions in which increased angiogenesis is desired (ischaemic tissues and peripheral vascular disease) and theoretically may have broader impact, although efficacy has yet to be shown.

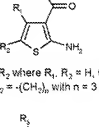
2-Aminothiophenes may not be ideal drug candidates due to their instability. The future for allosteric modulation of the adenosine A_1 receptor seems to lie in the discovery of new lead compounds. Particularly interesting is the disclosure by Chordia *et al.* [32] of 2-aminothiazoles, which represent a new class of such derivatives. These compounds lack the 3-aroyl moiety thought to be necessary for the allosteric enhancer activity of 2-aminothiophenes. The only structural features that 2-aminothiazoles appear to share with the 2-amino-3-aroylthiophenes is a five membered sulfur containing ring and an exocyclic amine.



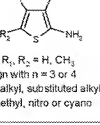
9 R_1 = R_2 where R_1, R_2 = H, CH_3
R_1, R_2 = -$(CH_2)_n$- with n = 3 or 4
R_3, R_4 = -(CH=CH)$_2$-
R_5 = R_6 = H
If R_6 is H, R_5 is halogen, alkyl, substituted alkyl, aryl, amino, trifluoromethyl, nitro or cyano



10 R_1 = R_2 where R_1, R_2 = H, CH_3
R_1, R_2 = -$(CH_2)_n$- with n = 3 or 4



11 R_1 = R_2 where R_1, R_2 = H, CH_3
R_1, R_2 = -$(CH_2)_n$ with n = 3 or 4



12 R_1 = R_2 where R_1, R_2 = H, CH_3
R_1, R_2 = -$(CH_2)_n$n with n = 3 or 4
R_3 = halogen, alkyl, substituted alkyl, aryl, amino, trifluoromethyl, nitro or cyano

Figure 3. General structures of 2-amino-3-heteroaryl thiophenes.

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103. KING PHARM. RES. & DEV., INC. US6248774 (2001).

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